Spectroscopy and Electrochemistry of Ruthenium-Modified Nucleic Acids: Design of a Novel Metal-Binding Nucleoside

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Spectroscopy and Electrochemistry of Ruthenium-Modified Nucleic Acids: Design of a Novel Metal-Binding Nucleoside

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Electron transfer (ET) reactions through DNA[1] have been the subject of numerous investigations due to the implications for light-induced DNA damage[2] and the quest for understanding long-range ET events in biological molecules.[3] An important objective in this area continues to be the facile and site-specific incorporation of metal complexes into DNA. While recent work has focused on nucleobasic and nonnucleosidic sites for the attachment of high-potential complexes,[4] our efforts have concentrated on the ribose ring (to minimize structural perturbations) as the incorporation site for *both* high- and low-potential metal complexes.[5]

To this end, we have designed a novel chelating nucleoside (**1**) that enables the preparation of a series of metallonucleosides. We report the synthesis and spectral characteristics of a unique electron donor−acceptor pair prepared from **1** consisting of low- (**2**) and high-potential (**3**) metallonucleosides. Further, we describe the first example of a metal-containing oligonucleotide (**4**) prepared by solid-phase methods starting with the metal complex derivatized directly to a solid support.

The metal-binding nucleoside **1**, 5′-O-(4,4′-dimethoxytrityl)-2′-iminomethylpyridyl-2′-deoxyuridine, was readily prepared in situ by condensation of 5′-DMT protected 2′-amino-2′-deoxyuridine[6] with 2-pyridinecarboxaldehyde (Figure 1).[7] The ruthenated nucleosides **2** and **3** were prepared by subsequent addition of $Ru(acac)_2(CH_3CN)_2$[8] (acac = acetylacetonate) and $Ru(bpy)_2Cl_2$ (bpy = 2,2′-bipyridine) to **1**; they represent the range of metal complexes that can be inserted at the 2′-chelate site.

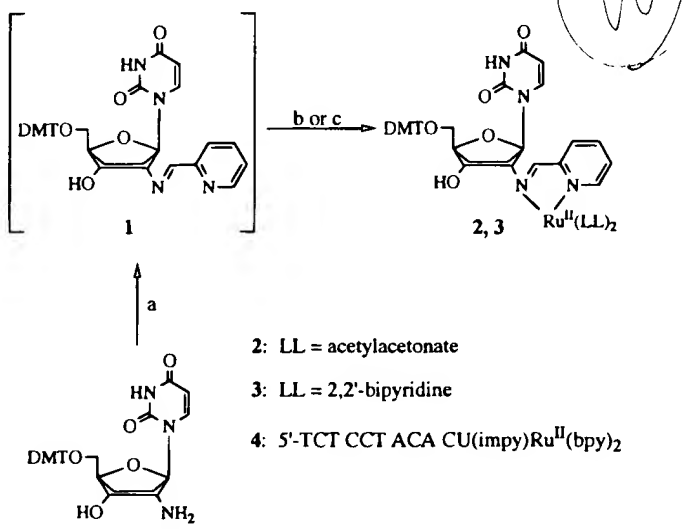

Figure 1. Synthesis and structures of 2′-ruthenated nucleosides and oligonucleotide **4**: (a) 2-pyridinecarboxaldehyde, EtOH, 2 h; (b) $Ru(acac)_2(CH_3CN)_2$, EtOH, 1 h, 79% yield; and (c) $Ru(bpy)_2Cl_2$, EtOH, 4 h, 19% yield.

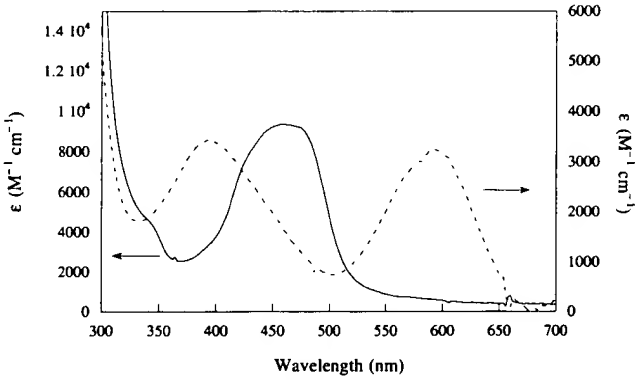

Figure 2. Absorption spectra of $[Ru(acac)_2(1)]$ (**2**) (dashed line) and $[Ru(bpy)_2(1)]^{2+}$ (**3**) (solid line) in ethanol and methanol, respectively.

Metallonucleoside **3** was selected for derivatization to the solid support due to its stability in both the mildly acidic and strongly basic solutions that are routinely encountered during automated DNA synthesis. Treatment of **3** with succinic anhydride produced the hemisuccinate form that was isolated by flash chromatography in 54% yield.[9] Derivatization of the solid support with succinated **3** enabled the preparation of oligonucleotide **4** with yields

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comparable to those observed in automated DNA synthesis. The duplex formed with **4** and its complementary sequence strand exhibited a single, cooperative melting transition with $T_m = 50$ °C (50 mM NaP_i, 0.5 M NaCl, pH 7.0; 2 °C higher than T_m of the corresponding unmodified duplex).

The spectral characteristics of metallonucleosides **2** and **3** are shown in Figure 2. The absorption spectrum of **2** reveals maxima at 396 ($\epsilon = 3600\ M^{-1}\ cm^{-1}$) and 592 nm ($\epsilon = 3400\ M^{-1}\ cm^{-1}$) in EtOH, which shift slightly in CH_2Cl_2 (392 nm, 3600 $M^{-1}\ cm^{-1}$; 602 nm, 3700 $M^{-1}\ cm^{-1}$). The reduction potentials of **2** and the model complex, $Ru(acac)_2(impy)$ (impy = iminomethylpyridine), are 92 and 33 mV vs Ag/AgCl, respectively. Apparently, the proximity of the nucleoside to the metal center causes the reduction potential to shift to more positive values.

The electronic spectra of **3** and **4** display a broad absorption band at 480 nm ($\epsilon = 9100\ M^{-1}\ cm^{-1}$), consistent with that previously reported by Keene and Meyer for $Ru(bpy)_2(impy)$.[10] This band, which is red-shifted from $\lambda_{max} = 452$ nm ($\epsilon = 14600\ M^{-1}\ cm^{-1}$) for $[Ru(bpy)_3]^{2+}$, reveals the effect of substituting iminomethylpyridine for bipyridine. Electrochemical measurements on **3** (in dichloromethane) and **4** (in water) give Ru(III/II) reduction potentials of 1.4 and 1.1 V vs Ag/AgCl, respectively.

Irradition of **3** and **4** at 480 nm yields identical lifetimes (λ_{max}(em) = 740 nm; $\tau = 42$ ns; $\Phi = 1.1 \times 10^{-4}$), which are shorter than that observed for $[Ru(bpy)_3]^{2+}$ (λ_{max}(em) = 625 nm; $\tau =$ 620 ns; $\Phi = 0.042$).[11] Indeed, the differences in lifetime and quantum yield correspond to an increase in the nonradiative rate constant (k_{nr}) from $1.54 \times 10^6\ s^{-1}$ for $[Ru(bpy)_3]^{2+}$ to $2.38 \times 10^7\ s^{-1}$ for **4**. The radiative rate constant (k_r) for **4** is on the order of $10^3\ s^{-1}$, suggesting that the emissive state is similar to other $Ru(bpy)_3^{2+}$-type chromophores. In comparison to $[Ru(bpy)_3]^{2+}$, the iminomethylpyridine ligand in $[Ru(bpy)_2(impy)]^{2+}$ is responsible for the red-shifted emission and the increase in k_{nr}.

We utilized resonance Raman (rR) spectroscopy to investigate the nature of the visible transitions of these complexes. The rR spectrum of **4** (identical to **3**; 441.6 nm excitation) is similar to that of other ruthenium polypyridyl compounds (Figure 3).[12] The peaks at 1023, 1173, 1276, 1316, 1488, 1552, and 1604 cm^{-1} correspond well to those observed in the rR spectrum of $[Ru(bpy)_3]^{2+}$.[13] Additional peaks (1242, 1471 cm^{-1}) represent excited-state distortions that are not present in $[Ru(bpy)_3]^{2+}$, and can be attributed to the impy ligand.

Despite the weak absorption of **2** at the available excitation frequencies (441.6 and 514.5 nm), rR spectroscopy provided valuable data. The rR spectrum (441.6 nm excitation) of **2** reveals

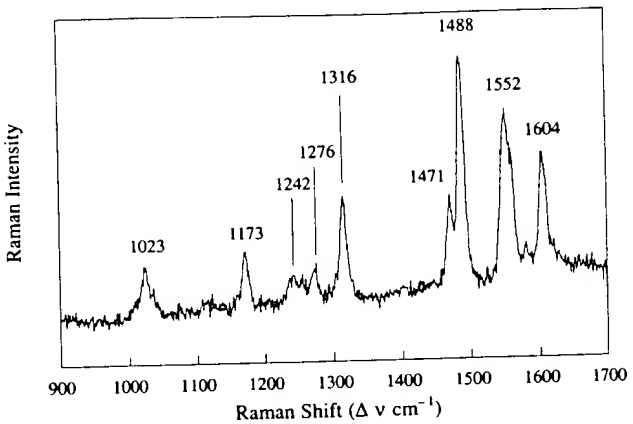

Figure 3. Resonance Raman spectrum of oligonucleotide **4** in unbuffered aqueous solution.

characteristic acac vibrations at 1528 (ν(CO)) and 1234 cm^{-1} (ν(C−C)).[14,15] Spectral features typically seen for polypyridyl complexes are absent, confirming the Ru $d\pi \rightarrow$ acac π^* nature of the transition.[16] The spectrum obtained with 514.5 nm excitation yields vibrations similar to those observed for complex **4** (1249, 1288, 1501, 1530, 1551, and 1597 cm^{-1}). As a result, the low-energy band at 592 nm is assigned as a Ru $d\pi \rightarrow$ impy π^* transition.

The synthesis of both the low-potential complex **2** and the metal-modified solid support represents a significant advance in the development of metal-containing oligonucleotides. The synthetic versatility of **1** is demonstrated by the preparation of **2** and **3**. Further, the distinct absorption and electrochemical features of these complexes are well-suited for ground-state ET studies involving DNA. Future work will examine these characteristics and focus on additional means of incorporating similar oxidants.

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Supporting Information Available: Instrumental details and experimental procedures for complexes **1**−**4** and $Ru(acac)_2(impy)$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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